MULTIFUNCTIONAL USE OF CASEIN AS RELEASE SYSTEM AND BLOCKING LAYER FOR IMPLANT COATINGS

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ABSTRACT

In situ tissue engineering requires to functionalize the surfaces of implants, to achieve better biocompatibility or to release pharmaceuticals. Often a burst release of such pharmaceuticals takes place and the surface functionality is gone rapidly. Casein-micelles (M-Cas) offer unique release capabilities for hydrophobic and hydrophilic substances. In this work, M-Cas were reconstructed and used as release system for curcumin as a model drug. Modified electrospun polycaprolactone (PCL)-fiber mats were coated with chitosan / tripolyphosphate nanoparticles (CS/TPP-NP) and functionalized with the reconstructed M-Cas. [1] CS/TPP-NPs and Na-caseinate (Cas) were used as blocking layer to slow down the release of curcumin loaded micelles. The layers were investigated by surface zeta-potential (ZP). The release of the curcumin from surfaces was possible and the blocking layers had a significant influence on the kinetics.

Keywords: casein-micelles, surface functionalization, blocking layers, controlled release, tissue engineering

INTRODUCTION

Drug release systems can be applied to implant surfaces, for example, by means of a layer-by-layer (LbL) coating. These systems often suffer from a burst release. Furthermore, the coating process may influence the structure and the release of the drugs. The interpenetration of the layers, which are subsequently applied to the surface, effects the release properties, because often a continues layer not separated ones is formed. In this large layer the substances are widely dispensed and probably released faster as if they had to overcome interfaces at different layers. [2, 3, 4]

Casein is a natural release system and offers the possibility of transporting hydrophilic and hydrophobic substances. [5] It is already used in some medical applications, as in the encapsulation of poorly soluble drugs and in bone tissue engineering as scaffold. Surface modification with casein was also proven feasible. However, working with casein or casein-micelles can be difficult due to preparation and purification steps. [5, 6]

Here we report on using reconstructed casein-micelles as release system for modified electrospun PCL-fiber mats and further adjustment of the release kinetics with additional blocking layers of Na-caseinate.

RESEARCH CONCEPT

Electrospun PCL-fiber mats were modified with CS-g-PCL and further coated with alginate. [1] In order to functionalize this primal scaffold with casein micelles, a positively charge surface was prepared with a layer of CS/TPP-NP. [3] The reconstructed micelles or Na-caseinate now could be coated on the scaffold and used in model system to release curcumin, investigate the release capacity and the blocking efficiency.

Reconstruction of Casein-Micelle

A dialysis method was used to reconstruct casein-micelles. Na-caseinate was dissolved in dest. water (50 mg/mL) and dialyzed (MWCO 3.5 kDa) against a 5 mM $CaCl_2$-solution. The reconstructed micelles were collected after 16 h and characterized by dynamic light scattering (DLS) (nano ZetaSizer ZS) for the size and zeta-potential. [7]

Encapsulation of Curcumin

Curcumin (1 mg/mL) was dispersed in an aqueous micelle solution (1 mg/mL) and was shaken (200 rpm) for 2 h. After an additional 1 h of resting, the loaded micelles in the supernatant were used in the release experiments. [8]

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Layer-by-Layer build up

In a dipping process CS/TPP-NPs (2 mg/mL) and Na-caseinate / casein-micelles (0.1 mg/mL - 5 mg/mL) were alternately coated onto a silicon-wafer. Each layer was dipped for 10 min and was washed 1 min with 0.1% of acetic acid (only CS/TPP layers) and water. The coatings were dried in a N_2-stream before the ellipsometry measurement. [3, 9]

Layer characterization

Surface zeta-potential measurements (SurPASS 3) of the casein layer on modified fiber mats were performed over a pH range from 9 to 2.

Release experiments

Micelles with encapsulated curcumin (1 mg/mL) were coated on modified fiber mats (8x16 mm). A second group was further coated with blocking layers (2 x (CS/TPP(1 mg/mL)-Cas(0,1 mg/mL))). The release was carried out over 6 h in a Na_2CO_3-buffer (1 mg/mL; pH 11), which is necessary to dissolve the curcumin and quantified by UV/Vis-analysis.

RESULTS

After 16 h of dialysis reconstructed Na-caseinate micelles had a size as z-average of 120 nm and a polydisperse index (PDI) of 0,224. The zeta potential at pH 7.4 was -19.1 mV. Longer dialysis time tend to result in smaller particles.

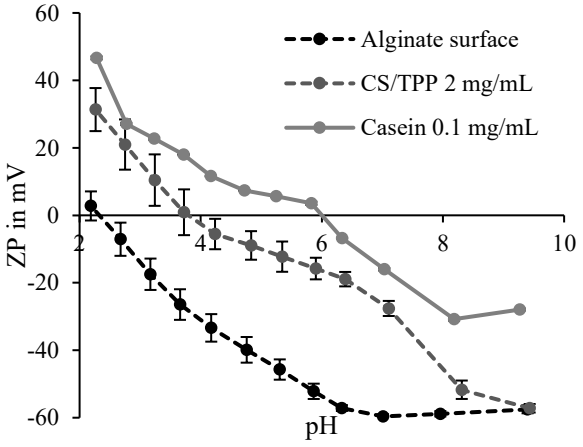

Fig. 1: Zetapotential measured as surface streaming potential measurements of modified PCL fiber mats, with different surface functionalization's, in a pH range from 9 to 2. A 1 mM KCl solution was used as electrolyte. Alginate is used as the negatively charged surface which is further coated with CS/TPP (1 mg/mL) and Na-caseinate (0.1 mg/mL).

The negative charge of reconstructed casein micelles and Na-caseinate itself makes them applicable in a layer-by-layer build-up alternating with positively charged CS/TPP nanogels. Various concentrations of casein were applied. A partial detachment of the casein layers was observed, while the next CS layer was coated. With less concentrated casein solution (0,1 mg/mL), no detachment was observed and the same thickness as after the detachment was reached.

The layers were further characterized by surface streaming potential measurements, shown in Fig. 1. The surface modifications manifest themselves in a change of the isoelectric point (IEP) as well as the general curve shape.

The release data for layers prepared with reconstructed casein micelles and the blocking capacity of additional Na-caseinate layers, is shown in Fig. 2.

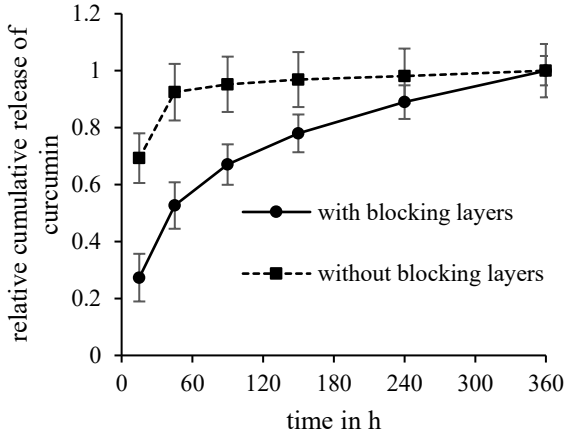

Fig. 2: Relative release of curcumin from fiber mats functionalized with reconstructed casein micelles at 37 °C in a Na_2CO_3 (pH 11), a) without blocking layers and b) with blocking layers (2 x (CS/TPP(1 mg/mL)-Cas(0,1 mg/mL))). The additional blocking layers showed a change in the release kinetics for curcumin.

DISCUSSION

A reconstruction of casein-micelles was possible with the dialysis method. Time, concentration of $CaCl_2$, type of dialysis tube and the temperature are all parameters determining the amount and velocity of the Ca^{2+} diffusion and therefore effect the micelle formation but all these factors can easily be set, and the reconstruction was well reproducible. The casein-micelles had a z-average size of ~120 nm, which is similar to natural micelles (~150 nm). The negative ZP of roughly -20 mV makes them applicable to interact with the positively charged CS/TPP-NP. [5]

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Curcumin was passively encapsulated by the micelles, after 2 h of shaking and 1 h resting the solution, the micelles had a yellow color. A rest of non-dissolved curcumin was still present at the bottom of the flask, meaning that not all of it could be encapsulated. By determining the undissolved curcumin, the encapsulation efficiency could be calculated. The supernatant of the curcumin loaded micelles were used to build up layers for the release experiments.

In the layer-by-layer method oppositely charged polyelectrolytes or particles are used to build-up the coating. While dipping in the CS/TPP-NP suspension the previously deposited casein layer was partly rinsed off and the layer thickness decreased somewhat. Using less concentrated casein solutions this effect was negligible, actually at a concentration of 0.1 mg/mL Na-caseinate and casein-micelles the thicknesses reached the same levels as without a rinse off effect. The LbL-method results in stable films because of the charge compensation of the two oppositely charged polyelectrolytes as well as the release of small counterions. [4] Furthermore, a certain degree of interpenetration of the layers occurs. In the case of higher casein concentrations besides the amount which is necessary to compensate the charge of the chitosan and to release the TPP ions, additional caseinate is adsorbed due to hydrophobic interactions, which are comparatively weak. However, this part is removed again when the substrate is dipped in the CS/TPP-NP dispersion. Interestingly the same effect occurred with the casein-micelles but in different extent. The layers in general were thicker, because of the Ca^{2+}-crosslinks within the micelles, but still a rinse off effect was observable. Similar explanations apply here. [9]

The coatings prepared by the LbL-method were characterized by surface streaming potential measurements (Fig1). The fundamental alginate layer, used as substrate for further coatings on the fiber mats [1] showed a typical curve shape for acid surfaces, where the zeta-potential rises but stays negative until very low pH values. In contrast, the chitosan/TPP layer behaves atypical. If chitosan/TPP is added as top layer onto the alginate a different behavior is observed (s. Fig1). The zeta-potential is rising faster for the alginate layer, but still negative. Furthermore, a plateau is observed. Decreasing the pH decreases the negative zeta-potential and at as isoelectric point of approximately 4 the potential turns positive This value is somewhat lower than expected for chitosan, Zemljič et. al. [10] reported an IEP of around pH 8. This can be reasoned to be a consequence of the interpenetration of the alginate and chitosan layers, resulting in a mixed layer. Similar observations were made by Sandri et. al. [11] for some chitosan systems and also attributed to the interaction / interpenetration of the layers, resulting in blended layers.

Finally, the casein (c = 0.1 mg/mL) layer was expected to show interpenetration and influences from the underlaying chitosan layer. However, for the casein layer the curve shape and isoelectric point (~6) fits to the literature IEP of the different caseins (~5-6) depending on the orientation and the casein on the surface. [12] With these results it can been assumed, that in this case the zeta potential is not influenced by the underlying chitosan and that there is no or only weak interpenetration. These are important findings for the use of the casein as blocking layers in a release system, because the casein builds a separated but attached layer. It can by hypothesized that the amphiphilic nature of the casein results in a layer with an inner hydrophobic phase, which being a barrier for the released drug.

Releasing curcumin from coated fiber mats with reconstructed casein micelles (1 mg/mL) as release system showed the burst release typical for this kind of release systems. [3] This changed markedly after the deposition of blocking layers (2 x (CS/TPP(1 mg/mL)-Cas(0,1 mg/mL))) on top of the micelles. The release of the curcumin slowed down significantly. The total release curcumin fluctuated between 3.2 µg and 4.6 µg, what strongly depends on the available surface. This proved that casein can be used as blocking layer and that reconstructed casein micelles are indeed a possible release system for hydrophobic substances.

CONCLUSIONS

In this work casein micelles were reconstructed with a dialysis method and were investigated for their use as release system for implant coatings. The direct use of casein-micelles is often not possible because of the requirement of separation and cleaning steps. The reconstructed micelles are more applicable. An encapsulation of curcumin as model drug was possible. Due to the negative charge of Na-caseinate and casein-micelles they can be used for surface modifications using the layer-by-layer method. Na-caseinate was used as blocking layer alternating with chitosan-tripolyphosphate nanoparticles. Surface zeta potential measurements indicated that the lower chitosan layer is not strongly interpenetrating the casein layer. The separation of the casein and the chitosan layers could provide different phases, which must be penetrated by the released substance. Indeed, the release of curcumin

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as a model drug from fiber mats could be slowed down significantly with these additional “blocking" layers, realizing a more sustained release. It can hypothesized that the inner hydrophobic sections of the casein layers play a major role in the release kinetics. In future studies, the layer interactions will be further analyzed as well as the importance of the hydrophobic character. Furthermore, the casein system will be applied for more relevant drug, like for examples signaling proteins.

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REFERENCES

[1] Cassan, Dominik de; Sydow, Steffen; Schmidt, Nadeschda; Behrens, Peter; Roger, Yvonne; Hoffmann, Andrea; Hoheisel, Anna Lena; Glasmacher, Birgit; Hänsch, Robert; Menzel, Henning (2018): Attachment of nanoparticulate drug-release systems on poly(ε-caprolactone) nanofibers via a graftpolymer as interlayer. In: Colloids Surf. B 163, S. 309-320. DOI: 10.1016/j.colsurfb.2017.12.050.

[2] Boudou, Thomas; Crouzier, Thomas; Ren, Kefeng; Blin, Guillaume; Picart, Catherine (2010): Multiple functionalities of polyelectrolyte multilayer films: new biomedical applications. In: Adv. Mater. 22 (4), S. 441-467. DOI: 10.1002/adma.200901327.

[3] Sydow, Steffen; Cassan, Dominik de; Hänsch, Robert; Gengenbach, Thomas R.; Easton, Christopher D.; Thissen, Helmut; Menzel, Henning (2019): Layer-by-layer deposition of chitosan nanoparticles as drug-release coatings for PCL nanofibers. In: Biomater. Sci. 7. DOI: 10.1039/c8bm00657a.

[4] Dercher, Gero; Schlenoff, Joseph B. (ed.) (2004): Multilayer Thin Films. In: Wiley-VCH. ISBN: 3527304401.

[5] Livney, Yoav D. (2010): Milk proteins as vehicles for bioactives. In: Curr. Opin. Colloid Interface Sci 15 (1-2), S 73-83. DOI: 10.1016/j.cocis.2009.11.002.

[6] Raveendran, Sreejith; Rochani, Ankit K.; Maekawa, Toru; Kumar, D. Sakthi (2017): Smart Carriers and Nanohealers: A Nanomedical Insight on Natural Polymers. In: Materials 10 (8), DOI: 10.3390/ma10080929.

[7] Picchio, Matias Luis; Cuggino, Julio César; Nagel, Gregor; Wedepohl, Stefanie; Minari, Roque Javier; Alvarez Igarzabal, Cecilia Inés; Gugliotta, Luis Marcelino; Calderón, Marcelo (2018): Crosslinked casein-based micelles as a dually responsive drug delivery system. In: Polym. Chem. 9 (25), S. 3499-3510. DOI: 10.1039/C8PY00600H.

[8] Pan, Kang; Luo, Yangchao; Gan, Yundi; Baek, Seung J.; Zhong, Qixin (2014): pH-driven encapsulation of curcumin in self-assembled casein nanoparticles for enhanced dispersibility and bioactivity. In: Soft Matter 10 (35), S. 6820-6830. DOI: 10.1039/c4sm00239c.

[9] Szyk-Warszyńska, Lilianna; Kilan, Katarzyna; Socha, Robert P. (2014): Characterization of casein and poly-L-arginine multilayer films. In: J. Colloid Interface Sci. 423, S. 76-84. DOI: 10.1016/j.jcis.2014.02.031.

[10] Zemljič, Lidija Fras; Plohl, Olivija; Vesel, Alenka; Luxbacher, Thomas; Potrč, Sanja (2020): Physicochemical Characterization of Packaging Foils Coated by Chitosan and Polyphenols Colloidal Formulations. In: Int. J. Mol. Sci., 21 (2). DOI: 10.3390/ijms21020495.

[11] Sandri, Giuseppina; Rossi, Silvia; Bonferoni, Maria Cristina; Miele, Dalila; Faccendini, Angela; Del Favero, Elena; Di Cola, Emanuela; Icaro Cornaglia, Antonia; Boselli, Cinzia; Luxbacher, Thomas; Malavasi, Lorenzo; Cantu', Laura; Ferrari, Franca (2019): Chitosan/glycosaminoglycan scaffolds for skin reparation. In; Carbohydr. Polym. 220, S. 219-227. DOI: 10.1016/j.carbpol.2019.05.069.

[12] Belitz, H.-D.; Grosch, Werner, Schieberle, Peter (2007): Lehrbuch der Lebensmittelchemie. Springer 6. Aufl. ISBN: 978-3-540-73201-3.